PATENT ABSTRACTS OF JAPAN

(11)Publication number : 07-198723
(43)Date of publication of application : 01.08.1995

(51)Int.Cl. G01N 33/576
C12N 15/09
C12P 21/02
// (C12N 15/09
C12R 1:92)
(C12P 21/02
C12R 1:19)

(21)Application number : 05-351227
(22)Date of filing : 29.12.1993

(71)Applicant : NIPPON ZEON CO LTD
(72)Inventor : ABE TAKAHARU
OKUDA HISASHI
NAGAYA ATSUSHI
YASUDA KANJI

(54) DIAGNOSING MEDICINE FOR HEPATITIS C-VIRUS INFECTIOUS DISEASE

(57)Abstract:
PURPOSE: To obtain a highly sensitive diagnosing medicine by bonding a fusion protein prepared by bonding a carrier protein to a hepatitis C-virus (HCV) antigen protein to hydrophilic particles.
CONSTITUTION: A diagnosing medicine for HCV infectious disease is obtained by bonding a fusion protein obtained by bonding a carrier protein to an HCV antigen protein to hydrophilic particles. The antigen protein is a structural or nonstructural protein. The structural protein includes envelope proteins, core proteins, etc., and the nonstructural protein includes NS1-NS5 proteins, polypeptide composed of 8 or more, preferably, 40 or more amino acids containing at least one epitope of these proteins. When a plurality of proteins is used in a combined way as the antigen protein, no limitation exists against the coupling order of the proteins. The fusion protein is prepared by coupling a carrier protein to the N-terminal side of the antigen protein.

LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The hepatitis C virus infectious disease diagnostic drug characterized by combining with a hydrophilic particle the fusion protein which connected the antigen protein and carrier protein of a hepatitis C virus, and changing.
[Claim 2] The hepatitis C virus infectious disease diagnostic drug according to claim 1 combined without furthermore the antigen protein of a hepatitis C virus minding carrier protein.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to a hepatitis C virus infectious disease diagnostic drug.
[0002]
[Description of the Prior Art] Viral hepatitis is liver disease which happens by infection of a hepatitis virus. There is a blood propagation mold hepatitis virus which is not A mold or B mold infected through blood besides A mold, B mold, and D (delta) mold as a hepatitis virus, either, and it is thought that this type of virus is what is classified into a recent-years C mold. The diagnostic drug of the hepatitis C virus (henceforth HCV) infectious disease by which current use is carried out is a diagnostic drug by the condensation method which combined HCV antigen protein with a latex particle or gelatin. Non-structural protein like structural protein like the envelope protein of HCV or core protein, NS1, or 5 protein as HCV antigen protein etc. is known. Generally, as a diagnostic drug of the virus which has envelopes, such as a FURABI virus, it is supposed that it is good to use what combined structural protein and non-structural protein as antigen protein (Proc.Natl.Acad.Sci.USA, 89, 10011-10015 (1992)), and the antigen protein which consists of core protein, NS3 protein, and NS4 protein also with the diagnostic drug of a HCV infectious disease is used. However, in order to prevent infection by transfusion etc. certainly, still it is not enough, and the diagnostic drug of higher sensibility is called for.
[0003]
[Problem(s) to be Solved by the Invention] As a result of inquiring wholeheartedly in order to obtain the condensation reagent for a HCV diagnosis with more high sensibility under this conventional technique, when the fusion protein which connected the antigen protein and carrier protein of HCV was combined with the hydrophilic particle, this invention persons find out that the antibody in HCV infectious disease patient's serum is detectable to high sensitivity, and came to complete this invention.
[0004]
[Means for Solving the Problem] According to this invention, the condensation reagent for a HCV diagnosis of the high sensitivity which combined with the hydrophilic particle the fusion protein which connected the antigen protein and carrier protein of HCV is offered in this way.
[0005] The antigen protein used by this invention is the well-known structural protein and the non-structural protein of HCV. The polypeptide (only henceforth an epitope) for which at least one epitope of these protein besides being NS1 protein, NS2 protein, NS3 protein, NS4 protein, NS5 protein, these protein, etc. is included as non-structural protein, such as envelope protein and core protein, as structural protein and which consists of 40 or more amino acid preferably is illustrated eight or more. The concrete example of such protein or an epitope is WO 90/No. 11069, WO 90/No. 14436, JP,4-004880,A, JP,5-81600,B, and Nucleic. Acid It is indicated by patent official reports and papers, such as Research, 17 (24) 10367-10372 (1989), this magazine, 18 (15) 4626 (1990), J.Virol., and 65(3)1105-1113 (1991). What combined core protein, NS3 protein, NS4 protein, and these epitope and them also in such antigen protein is desirable. The protein which has the 1st - the 583rd array among the amino acid sequences specifically indicated by the array number 1 of protein and JP,5-78395,A which have the amino acid sequence of array number 1 publication is illustrated. The amino acid to the 1st - 429th is NS3 protein, and the amino acid to the 430th - 767th of the amino acid sequence of array number 1 publication is NS4 protein. In order to acquire especially antigenic [with the high protein field which has the 430th - the 747th amino acid sequence in the 8th - 273rd or the 385th - 429th NS4 protein] in NS3 protein, it is important, and it is desirable to use antigen protein including these fields. Moreover, even if a part of amino acid sequence of antigen protein receives qualification by addition, a deficit, a permutation, omission, insertion, etc. of amino acid, as long as antigenic is equivalent to the protein which has an amino acid sequence said official report and given in a paper, although there is no limit, he can usually use it 90% or more preferably 80% or more, if homology with the amino acid sequence of an above-mentioned desirable protein field is 95% or more of thing still more

preferably. In addition, homology here makes an index what was measured by the DNA sequence input analysis system "DNASIS" (a putting on the market on the market agency: TAKARA SHUZO CO., LTD.).
[0006] What combined two or more protein as antigen protein When using (it is hereafter called conjugated protein), especially the sequence that protein is connected is not limited. For example, the thing which NS4 protein combined with the C terminal of NS3 protein, What NS3 protein combined with the C terminal of NS4 protein, the thing which NS3 protein combined with the C terminal of core protein, and NS4 protein combined with the C terminal of NS3 protein further, That by which core protein is inserted between NS3 protein and NS4 protein is illustrated.
[0007] The fusion protein used by this invention makes carrier protein connect with the amino terminal side of above-mentioned antigen protein further. Although it is not limited especially if carrier protein is a hydrophilic polypeptide thing It is the thing of 10,000-120,000 more preferably. desirable — molecular weight 2,000-500,000 — as an example of such protein Maltose binding protein (J. Biol.Chem., 259, 10606-10613 (1984)), Glutathione-S-transferase () and beta-galactosidase (it Gene(s)) 67, 31-40 (1988), etc. and its part are illustrated, especially, maltose binding protein is desirable and the maltose binding protein (henceforth MBP) of the Escherichia coli origin with a molecular weight of about 42kilodalton is more desirable. By making such carrier protein connect with above-mentioned antigen protein ** Since a proteinic hydrophilic property increases, it is [condensation] lifting-hard and it can be carried out. ** It becomes easy to show an antigen to a particle front face according to the spacer effectiveness of carrier protein, and reactivity with an antibody can be raised. ** There is effectiveness, like purification with the affinity chromatography using the support which made ligand carrier protein and the protein combined specifically becomes easy. When the carrier protein of the bacteria origin is especially used, since the manufacture effectiveness of the fusion protein in the gene engineering-technique using the bacteria used as the origin of carrier protein becomes high, it is desirable.
[0008] Although the fusion protein used by this invention may be obtained by what kind of approach, it can be obtained with the conventional method by the gene engineering-technique which is described below, for example. What is necessary is to carry out the transformation of the Escherichia coli etc. by the plasmid which has DNA (henceforth fusion protein DNA) which carries out the code of the fusion protein, to cultivate according to a conventional method, to make target protein discover, and just to isolate, in order to obtain the fusion protein which the antigen protein and carrier protein of the HCV origin have connected.
[0009] Fusion protein DNA consists of DNA which carries out the code of the antigen protein of the HCV origin, and DNA which carries out the code of the carrier protein. DNA (henceforth antigen protein DNA) which carries out the code of the antigen protein can be obtained from HCV infectious disease patient's serum according to conventional methods, such as the PCR method. DNA which carries out the code of the envelope protein indicated by said official report and paper as an example of antigen protein DNA DNA which carries out the code of (it is hereafter called env DNA) and the core protein DNA which carries out the code of (it is hereafter called core DNA) and the NS1 protein DNA which carries out the code of (it is hereafter called NS1 DNA) and the NS2 protein DNA which carries out the code of the NS3 protein which has the base sequence of array number (hereafter called NS2 DNA) 1 publication (It is hereafter called NS3 DNA), DNA (henceforth NS4 DNA) which carries out the code of the NS4 protein, DNA (henceforth NS5 DNA) which carries out the code of the NS5 protein, DNA which carries out the code of these epitopes are illustrated. These DNA may be mutated automatically or artificially, as long as the conditions of the above-mentioned antigen protein are fulfilled. Such DNA is core from 5' upstream on a natural HCV genome. DNA-env DNA-NS1 DNA-NS2 DNA, NS3 DNA-NS4 DNA-NS5 It exists as a series of things in the order of DNA. For this reason, in using DNA of the same configuration as a natural HCV genome as antigen protein DNA, there is especially no problem, but after a restriction enzyme etc. cuts DNA, it is necessary using a suitable linker to connect a required DNA field with a conventional method, to use what changed the sequence that the case where a part of each DNA is used, and each DNA were located in a line so that a reading frame may not shift. Furthermore, DNA which carries out the code of the carrier protein marketed is inserted in the plasmid which carried out cloning of the DNA of such antigen protein according to the conventional method using a suitable linker, and the plasmid which has DNA which carries out the code of the fusion protein used by this invention is obtained.
[0010] The Escherichia coli with which the bacillus which carries out a transformation by the plasmid which has fusion protein DNA is generally used, a Bacillus subtilis, yeast, etc. are illustrated, and the plasmid which inserts fusion protein DNA should just use a thing introducible into the bacillus which carries out a transformation. When MBP of the Escherichia coli origin is chosen as carrier protein, it is desirable to use the system of Escherichia coli from the point of manifestation effectiveness. As an example of Escherichia coli, JM109, TB1, etc. mention and it is ****. After cultivating the obtained transformation bacillus on suitable conditions and making fusion protein discover, it can be crushed by conventional methods, such as an approach using a supersonic wave, and an approach using a French press, a cell-free extract can be obtained, and the fusion protein which is isolated

and refined by approaches, such as a chromatography using the others, ion exchange resin, and the hydrophobic resin using amylose resin (product made from New England Biolabs) etc., ammonium sulfate fractionation, and electrophoresis, and targets this extract can be obtained. [affinity chromatography] What is necessary is just to usually cultivate them at 37 degrees C for 2 to 36 hours in the case of transformation Escherichia coli, although the culture conditions of a transformation bacillus will not be limited especially if they are conditions which a bacillus grows.

[0011] The hydrophilic particle used by this invention especially has desirable gelatin, although the amino acid system polymer indicated by the magazine "surface" 28th volume 791 pages (1991 issue) besides the hydrophilic latex particle of the acrylamide system indicated by JP,1-163663,A and JP,1-315408,A, and gelatin and an amino acid polymer system particle like the polymer of an amino acid carboxy anhydride is illustrated. When it is manufactured with a conventional method, for example, the coacervation method, and gelatin is used as a raw material, as for these, it is desirable to manufacture by the complex coacervation method which used polysaccharide, such as gum arabic, together. In order to hold the configuration of a particle furthermore, fixing by cross linking agents, such as glutaraldehyde, is desirable. In number average particle diameter, the particle size of a particle is an about 1-15-micrometer thing, and is an an average of 2-6-micrometer thing preferably. Moreover, the sharp thing of particle size distribution is good.

[0012] That what is necessary is just to perform the approach of combining above-mentioned fusion protein with the front face of an above-mentioned hydrophilic particle, by the same approach as the case where the conventional latex particle is used for example, the approach (it Johnson(s) H. — M. —) of carrying out condensation by the carbodiimide Although the approach (S. 6 Avrameas, B.Tandon work, Immunochemistry, 67 (1969)) of constructing a bridge by KBrenner, H.E.Hall work, J.Immunol., 97,701 (1966), and glutaraldehyde etc. is mentioned It is the approach of combining fusion protein with a particle by the condensation reaction using a 1-ethyl-3-(3-dimethylamino prolyl) carbodiimide preferably. what the C terminal side of all fusion protein combined with the particle similarly although the amino terminal side of all fusion protein did not necessarily combine with a particle the particle (it may be hereafter called an antigen sensitization particle) obtained in such an approach — it is not, either — a certain amount of probability — an amino terminal side — a particle — even joining together — if it is, it is available as a diagnostic drug.

[0013] The protein combined with a particle does not need to be only above-mentioned fusion protein, and may be used together with the structural protein and the non-structural protein of HCV. What was illustrated previously is mentioned as the structural protein used here or non-structural protein (henceforth HCV protein), and these do not need to connect with carrier protein. It uses as independent protein, and also these protein may be used as HCV compound antigen protein which connected two or more HCV protein. Moreover, even if HCV protein is protein of the same class as the antigen protein which constitutes fusion protein, it may be protein of a different class. the mixing ratio of the fusion protein combined with a particle, and HCV protein — 1 / 100 - 100/1 — it is 1 / 10 - 10/1 preferably. Since a nonspecific reaction occurs and the detection sensitivity as a diagnostic drug falls in the condition that there is only a certain protein so much, it is not desirable.

[0014] Furthermore, it is desirable to color a particle, when using this antigen sensitization particle for an immunological diagnostic drug, for example, passive agglutination. The particle of this invention which has a hydrophilic property is usually white, and the judgment of a condensation image can be made easy by coloring this. As a coloring agent, blue coloring matter, such as red dyes, such as rear KUTEBU violet, food red No.3, a rose bengal, and neutral red, or a crystal violet, and a methylene blue, etc. can be used, for example.

[0015] The desirable mode of the hepatitis C virus infectious disease diagnostic drug of this invention is shown below.

(1) The hepatitis C virus infectious disease diagnostic drug of this invention whose carrier protein is maltose binding protein, a glutathione-S-transferase, and one protein with which it is chosen out of the beta-galactosidase.

(2) The hepatitis C virus infectious disease diagnostic drug of this invention whose carrier protein is maltose binding protein of the Escherichia coli origin.

(3) The hepatitis C virus infectious disease diagnostic drug of this invention whose antigen protein is NS3 protein and/or NS4 protein.

(4) The hepatitis C virus infectious disease diagnostic drug of this invention whose hydrophilic particle is an amino acid polymer system particle.

(5) The hepatitis C virus infectious disease diagnostic drug of this invention whose antigen protein of the hepatitis C virus which has not connected carrier protein is core protein.

[0016]

[Effect of the Invention] The HCV infectious disease diagnostic drug of high sensitivity is obtained by combining

a hydrophilic particle and the antigen protein of HCV through carrier protein.
[0017]
[Example] An example is given to below and this invention is explained to it still more concretely. In addition, % in an example, the example of reference, the example of a trial, and the example of a comparative study is weight criteria as long as there is no notice especially.
[0018] NS3 (Example 1 of reference) and the acquisition of a plasmid and the array analysis hepatitis B virus negative of DNA which have antigen protein DNA which consists of NS4 protein — 0.5ml of Homo sapiens blood serums of 100 or more units of GPT values — the GUANIJIUMU thiocyanate solution (4M GUANIJIUMU thiocyanate —) of the amount of 5 times 50mM(s) Tris-HCl (pH7.6), 10mM EDTA, 0.1M 2-mercapto methanol and 2% ZARUKOSHIRU were added, it carried out the phenol/chloroform extraction, and ethanol precipitation refined all RNA in a blood serum by using glycogen as a carrier.
[0019] cDNA of RNA previously obtained considering the random hexamer as a primer according to Okamoto's and others (Japan J.Exp.Med., 60 volumes, No. 3, the 167 - 177th page, 1990) approach was produced using the cDNA composition system (Boehringer Mannheim make). The PCR method was performed by having made this cDNA into the template, and the target DNA fragment was amplified. After phosphorizing the five prime end of the amplified DNA fragment by T-four polynucleotide kinase, it connected with pUC18 digested with the restriction enzyme SmaI, and cloning was carried out. The Sequenase sequence kit (product made from United States Biochemical) determined the base sequence of cDNA of HCV by which cloning was carried out to each obtained plasmid. Consequently, NS3 made into the purpose from the acquired base sequence DNA and NS4 DNA was inserted in the SmaI site of a plasmid pUC18 according to the conventional method, and Plasmid pIB (4989bp) was built. The base sequence of antigen protein DNA of HCV inserted in this plasmid and a corresponding amino acid sequence are indicated by the array number 1.
[0020] (Example 2 of reference) MBP The DNA fragment (5' GTTGCGGAATTCGTGGACTTC) was inserted in 5' side of antigen protein DNA in the plasmid pIB built in the example 1 of the manufacture above-mentioned reference of the fusion protein which DNA, NS3, and NS4 protein DNA connected, and the EcoRI site was added. Moreover, the DNA fragment (5' ACGCGCCGAAGCTTAGTCGCTC) was similarly inserted in 3' side of antigen protein DNA, and the termination codon and the HindIII site were added. Thus, the plasmid of obtained 4955bp(s) was named pIB'.
[0021] This pIB' was digested with restriction enzymes EcoRI and HindIII, and the fragment (2250bp) of antigen protein DNA with which both ends were changed was obtained. On the other hand, plasmid pMAL-cRI (product made from New EnglandBiolabs) which has MBP is digested with restriction enzymes EcoRI and HindIII, and it is MBP. The fragment of 6101bp(s) containing DNA was obtained. The fragment and MBP of antigen protein DNA which were changed The DNA fragment containing DNA was connected by DNA ligase, and the obtained plasmid was named pMAL-IB. This plasmid is MBP to 5' side of the 19th - 2265th DNA of DNA shown in the array number 1. It has the DNA field which malE**2-26 (gene of the protein with which the 2-26th amino acid (transit peptide) of the maltose binding protein of the Escherichia coli origin suffered a loss) of DNA connected.
[0022] Thus, the transformation of 109 shares of Escherichia coli JM was carried out by obtained pMAL-IB, and the transformation bacillus of ampicillin resistance was obtained. Inoculation of this transformation bacillus was carried out so that the turbidity in 660nm might be set to 0.15 to 8I. ("Molecular Cloning"68 page) of LB culture media which added 50microg [/ml] ampicillin. This was guided by 1mM isopropyl-beta-D(-)-thio galactopyranoside after 2-hour shaking culture at 37 degrees C, and shaking culture was carried out at 37 more degrees C for 4 hours. The 20g wet fungus body was obtained according to the centrifugal separation of 15,000xg. It crushed by the French press after suspending this in the 60ml tris hydrochloric-acid buffer solution (25 mM, pH8.0), and supernatant liquid was collected by 15,000xg and the centrifugal separation for 20 minutes. This is made to stick to DEAE-Toyo Perl's (TOSOH CORP. make) column (diameter [of 2.5cm] x height of 36cm), and it is 0M-0.8M. Elution was performed by the concentration gradient (800ml) of NaCl, and protein MAL-IB made into the purpose was obtained. Furthermore, it was made to stick to the column (diameter [of 2.5cm] x height of 15cm) of amylose resin (product made from New England Biolabs), elution was performed by the maltose (PBS solution) of 10mM, and about 40mg of fusion protein which purity makes 99% or more of the purpose was obtained. When the concentration of this protein solution by which elution was carried out was measured with the Lowry method, it was 1.0mg/ml. It was used for actuation after this, having used this liquid as the antigen undiluted solution.
[0023] (Example 3 of reference) It used as the cutting back with restriction enzymes NdeI and ClaI, plasmid pIK4CE which has the gene which carries out the code of the NANBV antigen protein indicated by the manufacture JP,5-78395,A example 1 of core protein was used as the flush end by Klenow fragment, and the DNA fragments of 0.37Kbp were collected from agarose gel. This DNA fragment is a base sequence equivalent to the 1st to the 123rd amino acid sequence of the array which carries out the code of the part for the amino

terminal side 123 amino acid of the core protein of HCV, and was indicated by the array number 1 of JP,5-78395,A. It used as the cutting back with the restriction enzyme EcoRI, the plasmid pKK 223-3 (Pharmacia manufacture) was used as the flush end by Klenow fragment, the DNA fragment of 0.37kbp(s) collected previously was connected using DNA ligase, the transformation of 109 shares of Escherichia coli JM was carried out by the obtained plasmid, and the DNA fragment of 0.37Kbp chose the colony which has the plasmid pKKCla inserted in the forward direction from the transformation bacillus of the ampicillin resistance which appeared. Plasmid pKKCla is a plasmid in which the DNA fragment of about 0.3 Kbp(s) appears, when restriction enzymes KpnI and HindIII cut.

[0024] Subsequently, inoculation of Escherichia coli JM109' which carried out the transformation to 8l. of LB culture media which added 50microg [/ml] ampicillin by Plasmid pKKCla was carried out so that the turbidity in 660nm might be set to 0.15. After carrying out shaking culture of this at 37 degrees C for 2 hours, it guided by 1mM isopropyl-beta-D(-)-thio galactopyranoside, and shaking culture was carried out at 37 more degrees C for 2 hours. The 20g wet fungus body was obtained according to the centrifugal separation of 15,000xg. This was crushed by the French press after suspending in 60ml PBS (10mM potassium phosphate buffer solution, 0.85% sodium chloride), at-long-intervals alignment separation was carried out for 15000x g or 20 minutes, and precipitate was collected. Next, this precipitate was suspended in the 60ml solubilization buffer solution (7M urea, 20mM JISUREI toll, the 1% triton X-100, 50mM tris hydrochloric acid (pH8.0)), the shaking was carried out at the room temperature after distribution by the ultrasonic crusher overnight, and crude-protein liquid was obtained as a meltable fraction. This crude-protein liquid was made to stick to the column (diameter [of 2cm] x height of 16cm) of CM-toe yaw PAL (TOSOH CORP. make), under 6M urea existence, elution was performed by the concentration gradient (400ml) of a 0.2M-0.8M sodium chloride water solution, and purity obtained about 10mg of core protein of 99% or more of HCV. After dialyzing this by PBS, when the concentration of core protein was measured with the Lowry method, it was 1.5mg/ml. This liquid was used as the antigen undiluted solution.

[0025] (Example 4 of reference) The PCR method was performed with the conventional method using primer (5) 5'GTTGCGGAATTCGTGGACTTC and primer (6) 5' GCGAAGCTTTTAGGACTGTCTGA by having made into the template the plasmid pIB (4989bp) obtained in the example 1 of manufacture reference of the fusion protein which MBP and NS3 protein connected, and the DNA fragment of 834bp was obtained. pMAL-cRI is digested with restriction enzymes EcoRI and HindIII like the example 2 of reference, and it is MBP. The fragment of 6101bp(s) containing DNA was obtained. MBP after digesting the fragment of DNA of 834bp(s) obtained previously with restriction enzymes EcoRI and HindIII It connected by the DNA fragment and DNA ligase containing DNA of 6101bp(s), the obtained plasmid was named pIB3, and the transformation of 109 shares of Escherichia coli JM was carried out by this plasmid pIB3. Subsequently, inoculation of this transformation bacillus was carried out to 8l. of LB culture media which added 50microg [/ml] ampicillin so that the turbidity in 660nm might be set to 0.15. After carrying out shaking culture of this at 37 degrees C for 2 hours, it guided by 1mM isopropyl-beta-D(-)-thio galactopyranoside, and shaking culture was carried out at 37 more degrees C for 2 hours. The 25g wet fungus body was obtained according to the centrifugal separation of 15,000xg. This was crushed by the French press after suspending in 75ml PBS (10mM potassium phosphate buffer solution, 0.85% sodium chloride), at-long-intervals alignment separation was carried out for 20000x g or 30 minutes, and supernatant liquid was obtained. This supernatant liquid was made to stick to DEAE-Toyo Perl's (TOSOH CORP. make) column (2.5cmx36cm), elution was performed by the concentration gradient (600ml) of a 0-0.6M sodium chloride water solution, and protein MAL-IB3 (fusion protein which NS3 protein has connected with the C terminal side of MBP) made into the purpose was obtained. Furthermore, it was made to stick to the column (diameter [of 2.5cm] x height of 15cm) of amylose resin (product made from New England Biolabs), elution was performed by the maltose (PBS solution) of 10mM, and purity obtained 99% or more of protein MAL-IB3 (75mg). When the concentration of a protein solution was measured with the Lowry method, it was 2.5mg/ml. This liquid was used as the antigen undiluted solution.

[0026] (Example 5 of reference) 30ml (the amount of MAL-IB3 fusion protein is 75mg) of antigen undiluted solutions obtained in the example 4 of manufacture reference of NS3 protein was added, and the factor Xa solution (product made from New England Biolabs) was put [undiluted solutions] for 250microl and 120micro of 1 calcium chloride water solutions l of M at the room temperature overnight. Then, it prepared so that a sodium chloride might be dissolved in this and the concentration of a sodium chloride might be set to 2M. This was made to stick to phenyl-Toyo Perl's (TOSOH CORP. make) column (diameter [of 1.5cm] x height of 10cm). Elution was performed by the concentration gradient (100ml) of 2M-0M sodium chloride, and protein IB3 of 99% or more of purity was obtained. When this protein solution was measured with the Lowry method, it was 1.8ml/ml. This liquid was used as the antigen undiluted solution.

[0027] (Example 6 of reference) The PCR method was performed with the conventional method using primer (7) 5'CTCACTGAATTCGATGCCCAC and primer (8) 5' ACGCGCCGAAGCTTAGTCGCT by having made into the

template the plasmid pIB (4989bp) obtained in the example 1 of manufacture reference of the fusion protein which MBP and NS4 protein connected, and the DNA fragment of 1137bp was obtained. pMAL-cRI is digested with restriction enzymes EcoRI and HindIII like the example 2 of reference, and it is MBP. The fragment of 6101bp(s) containing DNA was obtained. MBP after digesting the fragment of DNA of 1137bp(s) obtained previously with restriction enzymes EcoRI and HindIII It connected by the DNA fragment and DNA ligase containing DNA of 6101bp(s), the obtained plasmid was named pIB4, and the transformation of 109 shares of Escherichia coli JM was carried out by this plasmid pIB4. Subsequently, inoculation of this transformation bacillus was carried out to 7.5l. of LB culture media which added 50microg [/ml] ampicillin so that the turbidity in 660nm might be set to 0.15. After carrying out shaking culture of this at 37 degrees C for 2 hours, it guided by 1mM isopropyl-beta-D(-)-thio galactopyranoside, and shaking culture was carried out at 37 more degrees C for 2 hours. The 20g wet fungus body was obtained according to the centrifugal separation of 15,000xg. This was crushed by the French press after suspending in 60ml PBS (10mM potassium phosphate buffer solution, 0.85% sodium chloride), at-long-intervals alignment separation was carried out for 25000x g or 60 minutes, and precipitate was collected. This precipitate was suspended in the 40ml solubilization buffer solution (7M urea, 20mM JISUREI toll, the 1% triton X-100, 50mM tris hydrochloric acid (pH8.0)), the shaking was carried out at the room temperature after distribution by the ultrasonic crusher overnight, and crude-protein liquid was obtained as a meltable fraction. After removing a urea from this crude-protein liquid by dialysis, it was made to stick to the column (diameter [of 2.5cm] x height of 15cm) of amylose resin (product made from New England Biolabs), elution was performed by the maltose (PBS solution) of 10mM, and purity obtained 99% or more of protein MAL-IB4 (70mg). When the concentration of a protein solution was measured with the Lowry method, it was 1.5mg/ml. This liquid was used as the antigen undiluted solution.

[0028] (Example 7 of reference) The manufacture isoelectric point (pI) of an amino acid polymer particle made water suspend the gelatin of 8.8, and warms and dissolved it in it. This liquid was adjusted to pH8.5 using the sodium-hydroxide water solution, water was added, and 5% of gelatin solution was obtained. Moreover, gum arabic was dissolved in water and 4% of gum arabic water solution was obtained by filtering. Two obtained water solutions were warmed at 40 degrees C, and it mixed 10ml at a time, respectively, could flow into 60ml of 40% methanol water solutions warmed at 40 degrees C, and stirred. Then, the acetic acid was dropped, pH was lowered even to about 4, and the particle was made to form. The obtained particle dispersion liquid were ice-cooled, 0.5g glutaraldehyde was added, and it stirred well. After putting at 4 degrees C one whole day and night, centrifugal was carried out for 10 minutes by 900xg, and particles were collected. After washing the collected particle with water enough by centrifugal actuation, it distributed in the formaldehyde water solution 4%, and it was put for five days at 4 degrees C. Centrifugal [of this] was carried out by 900xg, particles were collected, and it washed with water by centrifugal actuation. Thus, the number average particle diameter of the obtained particle was about 4 micrometers.

[0029] (Example 1) The diluent which added water to the particle (henceforth a support particle) obtained in the example 7 of preparation reference of the antigen sensitization particle A, adjusted dispersion liquid 10%, added the phosphate buffer solution to the antigen undiluted solution obtained in the example 2 of reference, and was diluted [ml] in 50microg /was adjusted. 1ml of 50microg [/ml] diluents of an antigen and 0.05ml of 1-ethyl-3-(3-dimethylamino prolyl) carbodiimide water solutions adjusted [ml] in 50mg /were added to 0.5ml of 10% support particle dispersion liquid. After shaking this at 37 degrees C for 1.5 hours, 2ml (the 0.1M potassium phosphate buffer solution (pH6.5), 1% normal rabbit serum, and 0.05%NaN3) of liquid for particle suspension prepared previously washed 3 times, finally it suspended in the 4ml liquid for particle suspension, and 1.25% of antigen sensitization particle A was obtained.

[0030] (Example 2) the antigen undiluted solution obtained in the examples 2 and 3 of preparation reference of the antigen sensitization particle B — respectively — a 20 or 10microg [/ml] ** — 1.25% of antigen sensitization particle B was obtained by the same approach as an example 1 except ********.

[0031] (Example 3) the antigen undiluted solution obtained in the examples 3, 4, and 6 of preparation reference of the antigen sensitization particle C — respectively — 20 and a 10 or 10microg [/ml] ** — 1.25% of antigen sensitization particle C was obtained by the same approach as an example 1 except ********.

[0032] (Example 4) the antigen undiluted solution obtained in the examples 3, 5, and 6 of preparation reference of the antigen sensitization particle D — respectively — 20 and a 5 or 10microg [/ml] ** — 1.25% of antigen sensitization particle D was obtained by the same approach as an example 1 except ********.

[0033] The sensitization particle A obtained in the example 1 as a reactant examination diagnostic drug particle with the HCV infection patient's serum by the microtiter technique (Example of a trial) the particle (core protein —) of the trade name "the OSO HCV Ab PA test II" by B, C and D, and Ortho Diagnostic Systems, Inc. Below thing; that is combining with the gelatin particle the HCV antigen protein which consists of some of NS3 protein and NS4 protein a table — including — "a commercial item" — saying — using — OSO by Ortho Diagnostic

Systems, Inc. HCV Ab According to the assay manual of the PA test II, the trial by the microtiter technique at the time of using each particle was performed. As for the thing and − by which, as for front Naka +, condensation was observed, condensation is not observed. A result is shown in Table 1.

[0034]

[Table 1]

C型肝炎患者 血清の希釈度	抗原感作粒子 A	抗原感作粒子 B	抗原感作粒子 C	抗原感作粒子 D	比較例 市販品
1／8192	+	+	+	+	+
1／16384	+	+	+	+	+
1／32768	+	+	+	+	−
1／65536	+	+	+	+	−
1／131072	+	+	+	+	−
1／262144	−	+	+	+	−
1／524288	−	−	−	−	−

[0035] The result of Table 1 showed that the antigen sensitization particle of this invention was excellent in the sensitivity as a diagnostic drug compared with a commercial item. In addition, when each antigen sensitization particle and example of a comparison used the forward Tsuneto blood serum instead of the specimen diluent as a control experiment, the condensation image was not observed at all in which blood serum dilution scale factor.

[0036] array number: — die-length [of one array]: — mold [of 2303 arrays]: — number [of nucleic-acid chains]: — double strand topology: — class [of straight chain-like array]: — Genomic DNA origin living thing name: — blood propagation mold non-A non-B hepatitis virus array TGC ACC CGG GGG GTT GCG AAG GCG GTG GAC TTC ATA CCC GTT GAG CCT 48 Cys Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Pro 1 5 10 15 ATG GAA ACT ACT ATG CGG TCT CCG GTC TTC ACA GAC AAC TCT TCC CCC 96 Met Glu ThrThr Met Arg Ser Pro Val Phe Thr Asp AsnSer Ser Pro 20 25 30 CCG GCT GTA CCG CAG ACATTC CAA GTG GCC CATCTA CAC GCT CCC ACT 144 Pro Ala Val Pro Gln Thr Phe Gln Val Ala His Leu His Ala Pro Thr 35 40 45GGC AGC GGTAAG AGC ACC AGAGTG CCA GCT GCA TAT GCC AGC CAA GGG 192 Gly Ser Gly Lys Ser Thr Arg Val Pro Ala Ala Tyr Ala Ser Gln Gly 50 55 60 TAC AAG GTG CTC GTC TTG AAC CCG TCCGTT GCC GCC ACA TTG GGC TTT 240 TyrLys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe 65 70 75 80 CGG GCG TAT ATG TCT AAA GCA CAT GGT ATC GAC CCC AAC ATC AGA ACT 288 Arg Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr 85 90 95 GGG GTG AGG ACT ATC ACC ACG GGT GCC CCT ATC ACA TAC TCC ACC TAC 336 Gly Val Arg Thr Ile Thr Thr Gly Ala Pro Ile Thr Tyr SerThr Tyr 100 105 110 GGC AAG TTC CTT GCC GACGGT GGA TGC TCC GGG GGC GCC TAT GAC ATC 384 Gly Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr AspIle 115 120 125ATC ATA TGTGAT GAG TGC CACTCA ACT GAC TCA ACT ACC ATC TTG GGC 432 Ile Ile Cys Asp Glu Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly 130 135 140 ATT GGC ACA GTC CTG GAC CAA GCG GAG ACG GCT GGA GCT CGG CTC GTC 480 Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val145 150 155 160 GTG CTC GCC ACC GCT ACG CCT CCG GGA TCG GTC ACC GTA CCA CAC CCC 528 Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro 165 170 175 AAT ATC GAG GAG GTG GCC CTG TCC AAC ACA GGA GAG ATT CCC TTC TAC 576 Asn Ile Glu Glu Val Ala Leu Ser Asn Thr Gly Glu Ile Pro Phe Tyr 180 185 190 GGC AAA GCC ATC CCC ATC GAG GTC ATC AAG GGG GGA AGT CAT CTC ATT 624 Gly Lys Ala Ile Pro Ile Glu Val Ile Lys Gly Gly Ser His Leu Ile 195 200 205TTC TGC CAT TCC AAG-AAG-AAG-TGT-GAC GAG CTC GCC GCA AAG-CTG-TCA 672Phe Cys His Ser Lys-Lys-Lys-Cys-Asp Glu Leu Ala Ala Lys Leu Ser 210 215 220GCC CTC GGA CTC AAT GCT GTA GCG TAT TAT CGG GGT CTT GAT GTG TCC 720 Ala Leu Gly Leu Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser225 230 235 240 GTC ATA CCG ACC AGC GGA GAC GTC GTC GTC GTG GCGACAGAC GCT CTA 768 Val Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu 245 250 255 ATG ACG GGC TAC ACC GGC GAC TTT GACTCAGTG ATC GAC TGT AAC ACA 816 Met Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp CysAsn Thr 260 265 270 TGT GTC ATC CAG ACA GTCGAT TTT AGT TTG GAT CCC ACT TTC ACC ATC 864 Cys Val Ile Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe ThrIle 275 280 285GAG ACA ACGACC GTG CCC CAAGAC GCG GTG TCG CAC CCG CAA CGG CGA 912 Glu Thr Thr Thr Val Pro Gln Asp Ala Val Ser His Pro Gln Arg Arg 290 295 300 GGT AGG ACT GGC AGA GGT AGG AGA GGC ATC TAC AGG TTT GTG ACT CCA 960 Gly Arg Thr Gly Arg Gly Arg Arg Gly Ile Tyr Arg Phe Val Thr Pro305 310 315 320GGA GAA CGG CCC TCG GGC

ATG TTC GAT TCT TCG GTC CTG TGT GAG TGC 1008 Gly Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys 325 330 335TAT GAC GCA GGC TGT GCT TGG TAC GAG CTC ACG CCC GCT GAG ACT TCA 1056 Tyr Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Ser 340 345 350GTT AGG TTA CGG GCT TAC CTG AAT ACA CCA GGT TTA CTC GTC TGT CAG 1104 Val Arg Leu Arg Ala Tyr Leu Asn Thr Pro Gly Leu Leu Val Cys Gln 355 360 365GAC CAT CTG GAG TTC TGG GAG GGT GTC TTC ACA GGC CTC ACT CAT ATA 1152 Asp His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile 370 375 380GAT GCC CAC TTC TTG TCT CAG ACT AAG CAA GCA GGA GAC AGC TTC CCC 1200 Asp Ala His Phe LeuSer Gln Thr Lys Gln Ala Gly Asp Ser Phe Pro385 390 395 400TAC CTG GTA GCATAC CAG GCT ACA GTG TGC GCC AGG GCC CAA GCT CTA 1248 Tyr Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Leu 405 410 415CCT CCA TCG TGG GAT CAA-ATG-TGG-AAG-TGT CTC ACA CGG CTA AAG CCT 1296Pro Pro-Ser-Trp-Asp-Gln-Met Trp Lys Cys Leu Thr-Arg-Leu-Lys-Pro 420 425 430ACG CTA ACG CGG CCA ACA CCC CTG TTG TAT AGG CTA GGA GCT GTG CAA 1344 Thr Leu Thr Arg Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln 435 440 445AAC GAG GTC ACC CTC ACA CAC CCC GTA ACC AAA TAC ATC ATG GCA TGC 1392 Asn Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Ala Cys 450 455 460ATG TCA GCT GAC CTA GAG ATC GTC ACT AGC ACC TGG GTG CTG GTA GGC 1440 Met Ser Ala Asp LeuGlu Ile Val Thr Ser Thr Trp Val Leu Val Gly465 470 475 480GGG GTC CTT GCCGCT CTG GCC GCG TAC TGC CTG ACA ACG GGC AGC GTG 1488 Gly Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Thr Thr Gly Ser Val 485 490 495GTC ATT GTG GGC AGG GTC GTC TTG TCA GGG AGG CCG GCT ATC ATT CCC 1536 Val Ile Val Gly Arg Val Val Leu Ser Gly Arg Pro Ala Ile Ile Pro 500 505 510GAC AGG GAA GTT CTC TAC CGG GAG TTC GAC GAG ATG GAG GAG TGC GCC 1584 Asp Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys Ala 515 520 525ACA CAC CTC CCT TAC ATC GAA CAG GGA ATG CAG CTC GCC GAG CAA TTC 1632 Thr His Leu Pro Tyr Ile Glu Gln Gly Met Gln Leu Ala Glu Gln Phe 530 535 540AAG CAG AAG GCG TTC GGG TTG CTG CAA ACA GCC ACC AAA CAA GCG GAG 1680 Lys Gln Lys Ala PheGly Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu545 550 555 560GCT GCT GCT CCCGTG GTG GAG TCC AAG TGG CGG ACC CTT GAG GCT TTC 1728 Ala Ala Ala Pro Val Val Glu Ser Lys Trp Arg Thr Leu Glu Ala Phe 565 570 575TGG GCG AAG CAC ATG TGG AAT TTC ATC AGC GGG ATA CAA TAC TTG GCG 1776 Trp Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala 580 585 590GGC CTG TCG ACT CTG CCT GGG AAC CCC GCG ATA GCA TCG CTC ATG GCA 1824 Gly Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala 595 600 605TTC ACA GCC TCT ATC ACC AGC CCG CTC ACC ACC CAA CAC ACC CTC TTG 1872 Phe Thr Ala Ser Ile Thr Ser Pro Leu Thr Thr Gln His Thr Leu Leu 610 615 620TTT AAC ATC TTG GGG GGA-TGG-GTG-GCT-GCC CAA CTC GCC CCC ACC AGC 1920Phe Asn-Ile-Leu-Gly-Gly-Trp Val Ala Ala Gln Leu-Ala-Pro-Thr-Ser625 630 635 640GCT GCT TCA GCT TTC GTG GGC GCCGGC ATT GCC GGT GCG GCT GTT GGC 1968 Ala Ala Ser Ala Phe Val Gly Ala Gly Ile Ala Gly Ala Ala Val Gly 645 650 655AGC ATA GGC CTT GGG AAG GTG CTT GTG GAC ATT CTA GCG GGT TAT GGA 2016 Ser Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly 660 665 670GCG GGG GTG GCA GGC GCA CTC GTG GCC TTT AAG GTC ATG AGT GGT GAG 2064 Ala Gly Val Ala Gly Ala Leu Val Ala Phe Lys Val Met Ser Gly Glu 675 680 685ATG CCC TCC ACT GAG GAC CTG GTC AAC TTG CTC CCT GCT ATC CTC TCT 2112 Met Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Ser 690 695 700CCT GGT GCC CTG GTC GTC GGG GTC GTG TGC GCA GCA ATA CTG CGT CGG 2160 Pro Gly Ala Leu ValVal Gly Val Val Cys Ala Ala Ile Leu Arg Arg705 710 715 720CAT GTG GGC CCAGGG GAG GGG GCT GTG CAG TGG ATG AAC CGG CTG ATA 2208 His Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile 725 730 735GCG TTC GCT TCG CGG GGC AAC CAC GTC TCC CCC ACG CAC TAT GTG CCT 2256 Ala Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val Pro 740 745 750 GAG AGC GAC GCC GCA GCG CGC GTC ACC CAG ATC CTC TCC AGC CTT AC 2303 Glu Ser Asp Ala Ala Ala Arg Val Thr Gln Ile Leu Ser Ser Leu 755 760 765 767

[Translation done.]

(19)日本国特許庁（ＪＰ）　(12)公開特許公報（A）　(11)特許出願公開番号

特開平7－198723

(43)公開日　平成７年(1995)８月１日

(51)Int.Cl.[6]	識別記号	FI		
G01N 33/576	Z			
C12N 15/09	ZNA			
C12P 21/02	C 9282-4B			
	9281-4B	C12N 15/00	ZNA	A
		(C12N 15/00	ZNA	A

審査請求　未請求　請求項の数２　ＦＤ　（全10頁）　最終頁に続く

(21)出願番号　特願平5－351227

(22)出願日　平成５年(1993)12月29日

(71)出願人　000229117
日本ゼオン株式会社
東京都千代田区丸の内２丁目６番１号

(72)発明者　阿部　孝春
千葉県山武郡大網白里町上谷新田436－５

(72)発明者　奥田　尚志
神奈川県相模原市共和４－20－18

(72)発明者　長屋　敦
神奈川県横浜市港北区太尾町873

(72)発明者　安田　斡司
神奈川県茅ヶ崎市南湖６－９－33

(54)【発明の名称】Ｃ型肝炎ウイルス感染症診断薬

(57)【要約】
【目的】　高感度のＣ型肝炎ウイルス感染症診断薬を提供する。
【構成】　Ｃ型肝炎ウイルスの抗原タンパク質とキャリアタンパク質とを連結している融合タンパク質を親水性粒子に結合させてＣ型肝炎ウイルス感染症診断薬とする。

1

【特許請求の範囲】

【請求項1】　C型肝炎ウイルスの抗原タンパク質とキャリアタンパク質とを連結した融合タンパク質を親水性粒子に結合して成ることを特徴とするC型肝炎ウイルス感染症診断薬。

【請求項2】　さらにC型肝炎ウイルスの抗原タンパク質がキャリアタンパク質を介さずに結合している請求項1記載のC型肝炎ウイルス感染症診断薬。

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明は、C型肝炎ウイルス感染症診断薬に関する。

【0002】

【従来の技術】ウイルス性肝炎は肝炎ウイルスの感染により起こる肝疾患である。肝炎ウイルスとしてA型、B型、D（デルタ）型のほか、血液を介して感染するA型でもB型でもない血液伝播型肝炎ウイルスがあり、このタイプのウイルスは近年C型に分類されるものであると考えられている。現在使用されているC型肝炎ウイルス（以下、HCVという）感染症の診断薬は、ラテックス粒子やゼラチンにHCV抗原タンパク質を結合させた凝集法による診断薬である。HCV抗原タンパク質としては、HCVのエンベロープタンパク質やコアタンパク質のような構造タンパク質、NS1ないし5タンパク質のような非構造タンパク質などが知られている。一般に、フラビウイルスなどのエンベロープを有するウイルスの診断薬としては、抗原タンパク質として構造タンパク質と非構造タンパク質とを組み合わせたものを用いるのがよいとされており（Proc. Natl. Acad. Sci. USA, 89, 10011－10015（1992））、HCV感染症の診断薬でもコアタンパク質、NS3タンパク質、NS4タンパク質からなる抗原タンパク質が用いられている。しかし、輸血による感染などを確実に防ぐためにはまだ充分ではなく、より高い感度の診断薬が求められている。

【0003】

【発明が解決しようとする課題】本発明者らは、かかる従来技術のもとでより感度の高いHCV診断用凝集試薬を得るべく鋭意検討した結果、HCVの抗原タンパク質とキャリアタンパク質とを連結した融合タンパク質を親水性粒子に結合すると、高感度にHCV感染症患者血清中の抗体を検出できることを見いだし、本発明を完成するに至った。

【0004】

【課題を解決するための手段】かくして本発明によれば、HCVの抗原タンパク質とキャリアタンパク質とを連結した融合タンパク質を親水性粒子に結合させた高感度のHCV診断用凝集試薬が提供される。

【0005】本発明で用いる抗原タンパク質は、公知のHCVの構造タンパク質や非構造タンパク質である。構造タンパク質としては、エンベロープタンパク質、コアタンパク質など、非構造タンパク質としては、NS1タンパク質、NS2タンパク質、NS3タンパク質、NS4タンパク質、NS5タンパク質やこれらのタンパク質などのほか、またこれらのタンパク質の少なくともひとつのエピトープを含む8以上、好ましくは40以上のアミノ酸からなるポリペプチド（以下、単にエピトープという）なども例示される。このようなタンパク質やエピトープの具体的な例は、WO90／11069号、WO90／14436号、特開平4－004880号、特公平5－81600号、Nucleic Acid Research, 17（24）10367－10372（1989）、同雑誌, 18（15）4626（1990）、J. Virol., 65（3）1105－1113（1991）等の特許公報や論文により開示されている。このような抗原タンパク質のなかでもコアタンパク質、NS3タンパク質、NS4タンパク質やこれらのエピトープ及びそれらを組み合わせたものが好ましい。具体的には、配列番号1記載のアミノ酸配列を有するタンパク質や特開平5－78395号公報の配列番号1に記載されたアミノ酸配列のうち第1番目～第583番目の配列を有するタンパク質などが例示される。配列番号1記載のアミノ酸配列は、第1番目～第429番目までのアミノ酸がNS3タンパク質であり、第430番目～第767番目までのアミノ酸がNS4タンパク質である。特に、NS3タンパク質のなかで第8番目～第273番目や第385番目～第429番目、NS4タンパク質の中で第430番目～第747番目のアミノ酸配列を有するタンパク質領域が高い抗原性を得るためには重要であり、これらの領域を含む抗原タンパク質を用いることが好ましい。また、抗原タンパク質のアミノ酸配列の一部がアミノ酸の付加・欠損・置換・脱落・挿入などで修飾を受けたものであっても、抗原性が前記公報及び論文記載のアミノ酸配列を有するタンパク質と同等である限り制限はないが、通常、上述の好ましいタンパク質領域のアミノ酸配列との相同性が80％以上、好ましくは90％以上、更に好ましくは95％以上のものであれば使用することができる。尚、ここでいう相同性は、DNAシーケンス入力解析システム「DNASIS」（発売元：宝酒造（株））により測定されたものを指標とするものである。

【0006】抗原タンパク質として複数のタンパク質を組み合わせたもの（以下、複合タンパク質という）を用いる場合、タンパク質の連結される順序は特に限定されず、例えば、NS3タンパク質のC末端にNS4タンパク質が結合したもの、NS4タンパク質のC末端にNS3タンパク質が結合したもの、コアタンパク質のC末端にNS3タンパク質が結合しさらにNS3タンパク質のC末端にNS4タンパク質が結合したもの、NS3タンパク質とNS4タンパク質の間にコアタンパク質が挿入

されているものなどが例示される。

【0007】本発明で使用する融合タンパク質は、上述の抗原タンパク質のN末端側に、更にキャリアタンパク質を連結させたものである。キャリアタンパク質は、親水性ポリペプチドものであれば特に限定されるものではないが、好ましくは分子量2，000～500，000、より好ましくは10，000～120，000のものであり、このようなタンパク質の具体例としては、マルトース結合タンパク質（J．Biol．Chem．、259、10606－10613（1984））、グルタチオンーSートランスフェラーゼ（）やβーガラクトシダーゼ（Gene、67、31－40（1988））などやその一部が例示され、なかでもマルトース結合タンパク質が好ましく、分子量約42キロダルトンの大腸菌由来のマルトース結合タンパク質（以下、MBPという）がより好ましい。このようなキャリアタンパク質を上述の抗原タンパク質に連結させることにより、①タンパク質の親水性が高まるため凝集を起こし難くすることができる、②キャリアタンパク質のスペーサー効果により抗原が粒子表面に呈示し易くなり抗体との反応性を高めることができる、③キャリアタンパク質と特異的に結合するタンパク質をリガンドとした担体を用いたアフィニティークロマトグラフィーでの精製が容易になる等の効果があり、とりわけ細菌由来のキャリアタンパク質を用いた場合、キャリアタンパク質の由来となる細菌を用いた遺伝子工学的手法での融合タンパク質の製造効率が高くなるので好ましい。

【0008】本発明で使用される融合タンパク質は、如何なる方法により得られたものであってもよいが、例えば以下に述べるような遺伝子工学的手法による常法により得ることができる。HCV由来の抗原タンパク質およびキャリアタンパク質が連結している融合タンパク質を得るためには、融合タンパク質をコードするDNA（以下、融合タンパク質DNAという）を有するプラスミドで大腸菌などを形質転換し、常法に従って培養し、目的とするタンパク質を発現させ、単離すればよい。

【0009】融合タンパク質DNAは、HCV由来の抗原タンパク質をコードするDNAとキャリアタンパク質をコードするDNAとから構成される。抗原タンパク質をコードするDNA（以下、抗原タンパク質DNAという）は、HCV感染症患者血清からPCR法などの常法にしたがって得ることができる。抗原タンパク質DNAの具体例としては、前記公報や論文に記載されたエンベロープタンパク質をコードするDNA（以下、env DNAという）、コアタンパク質をコードするDNA（以下、core DNAという）、NS1タンパク質をコードするDNA（以下、NS1 DNAという）、NS2タンパク質をコードするDNA（以下、NS2 DNAという）、配列番号1記載の塩基配列を有するNS3タンパク質をコードするDNA（以下、NS3 DNAという）、NS4タンパク質をコードするDNA（以下、NS4 DNAという）、NS5タンパク質をコードするDNA（以下、NS5 DNAという）やこれらのエピトープをコードするDNAなどが例示される。これらDNAは、前述の抗原タンパク質の条件を満たす限りにおいて、自然にまたは人工的に変異させられたものであってもよい。これらのDNAは、天然のHCVゲノム上では、5'上流側からcore DNA・env DNA・NS1 DNA・NS2 DNA・NS3 DNA・NS4 DNA・NS5 DNAの順で一連のものとして存在している。このため、天然のHCVゲノムと同じ構成のDNAを抗原タンパク質DNAとして用いる場合には、とくに問題はないが、各DNAの一部を使用する場合や各DNAの並ぶ順番を変えたものを使用する場合には、制限酵素などでDNAを切断した後、必要なDNA領域を読み枠がずれないように適当なリンカーを用いて、常法により連結する必要がある。さらに、このような抗原タンパク質のDNAを常法にしたがってクローニングしたプラスミドに、市販されているキャリアタンパク質をコードするDNAを適当なリンカーを用いて挿入し、本発明で使用する融合タンパク質をコードするDNAを有するプラスミドを得る。

【0010】融合タンパク質DNAを有するプラスミドで形質転換する菌は、一般に使用される大腸菌、枯草菌、酵母などが例示され、融合タンパク質DNAを挿入するプラスミドは形質転換する菌に移入することができるものを用いればよい。キャリアタンパク質として大腸菌由来のMBPを選択した場合には、発現効率の点から大腸菌の系を用いるのが好ましい。大腸菌の具体例としては、JM109やTB1などが挙げれる。得られた形質転換菌を適当な条件で培養して融合タンパク質を発現させた後、超音波を用いる方法やフレンチ・プレスを用いる方法などの常法により破砕されて無細胞抽出液を得、この抽出液をアミロース・レジン（New England Biolabs社製）などを用いたアフィニティ・クロマトグラフィーのほか、イオン交換樹脂や疎水性樹脂を用いたクロマトグラフィー、硫安分画、電気泳動等の方法によって単離・精製され、目的とする融合タンパク質を得ることができる。形質転換菌の培養条件は、菌が生育する条件であれば特に限定されないが、形質転換大腸菌の場合、通常37℃で2～36時間培養すればよい。

【0011】本発明で使用する親水性粒子は、特開平1－163663号公報や特開平1－315408号公報に記載されたアクリルアミド系の親水性ラテックス粒子、ゼラチンやアミノ酸カルボキシ無水物の重合体のようなアミノ酸ポリマー系粒子のほか、雑誌「表面」第28巻791頁（1991年発行）に記載されているアミノ酸系ポリマーが例示されるが、とりわけゼラチンが好ましい。これらは、常法、例えばコアセルベーション法

により製造され、ゼラチンを原料とした場合には、アラビアゴム等の多糖類を併用したコンプレックス・コアセルベーション法で製造するのが好ましい。さらに粒子の形状を保持するために、グルタルアルデヒド等の架橋剤により固定化することが好ましい。粒子の粒径は、数平均粒子径で１～１５μm程度のものであり、好ましくは平均２～６μmのものである。また、粒径分布はシャープなものがよい。

【００１２】上述の親水性粒子の表面に上述の融合タンパク質を結合させる方法は従来のラテックス粒子を用いた場合と同様の方法で行えばよく、例えば、カルボジイミドで縮合させる方法（H. M. Johnson、K. Brenner、H. E. Hall著、J. Immunol.、97、701（1966））、グルタルアルデヒドで架橋する方法（S. Avrameas、B. Tandonら著、Immunochemistry、6、67（1969））等が挙げられるが、好ましくは１－エチル－３－（３－ジメチルアミノプロリル）カルボジイミドを用いた縮合反応で粒子に融合タンパク質を結合させる方法である。この様な方法において得られる粒子（以下、抗原感作粒子ということがある）は、必ずしもすべての融合タンパク質のN末端側が粒子に結合したものであるとは限らないが、同様に全ての融合タンパク質のC末端側が粒子に結合したものでもなく、ある程度の確率でN末端側が粒子に結合してさえいれば診断薬として利用可能である。

【００１３】粒子に結合させるタンパク質は、上述の融合タンパク質のみである必要はなく、HCVの構造タンパク質や非構造タンパク質と併用してもよい。ここで用いられる構造タンパク質や非構造タンパク質（以下、HCVタンパク質という）としては、先に例示したものが挙げられ、これらはキャリアタンパク質と連結している必要はない。これらのタンパク質は単独のタンパク質として用いるほか、複数のHCVタンパク質を連結したHCV複合抗原タンパク質として用いてもよい。また、HCVタンパク質は、融合タンパク質を構成している抗原タンパク質と同じ種類のタンパク質であっても、異なる種類のタンパク質であってもよい。粒子に結合させる融合タンパク質とHCVタンパク質の混合比は、１／１００～１００／１、好ましくは１／１０～１０／１である。あるタンパク質のみが多量にある状態では、非特異的反応が起こり、診断薬としての検出感度が低下するので好ましくない。

【００１４】更に、この抗原感作粒子を免疫学的診断薬、例えば受身凝集反応に使用する場合、粒子を着色するのが好ましい。親水性を有する本発明の粒子は通常は白色であり、これを着色することで凝集像の判定を容易にすることができる。着色剤としては、例えば、リアクテブ・バイオレット、食用赤色３号、ローズベンガル、ニュートラルレッドなどの赤色色素、あるいはクリスタルバイオレット、メチレンブルーなどの青色色素等を用いることが出来る。

【００１５】本発明のC型肝炎ウイルス感染症診断薬の好ましい態様を以下に示す。

（１）　キャリアタンパク質がマルトース結合タンパク質、グルタチオン－Ｓ－トランスフェラーゼ、β－ガラクトシダーゼから選ばれるひとつのタンパク質である本発明のC型肝炎ウイルス感染症診断薬。

（２）　キャリアタンパク質が大腸菌由来のマルトース結合タンパク質である本発明のC型肝炎ウイルス感染症診断薬。

（３）　抗原タンパク質がＮＳ３タンパク質及び／またはＮＳ４タンパク質である本発明のC型肝炎ウイルス感染症診断薬。

（４）　親水性粒子がアミノ酸ポリマー系粒子である本発明のC型肝炎ウイルス感染症診断薬。

（５）　キャリアタンパク質の連結していないC型肝炎ウイルスの抗原タンパク質がコアタンパク質である本発明のC型肝炎ウイルス感染症診断薬。

【００１６】

【発明の効果】親水性粒子とHCVの抗原タンパク質とを、キャリアタンパク質を介して結合させることにより、高感度のHCV感染症診断薬が得られる。

【００１７】

【実施例】以下に実施例を挙げて本発明をさらに具体的に説明する。尚、実施例、参考例、試験例、および比較試験例中の％は特に断りのないかぎり重量基準である。

【００１８】（参考例１）ＮＳ３およびＮＳ４タンパク質からなる抗原タンパク質ＤＮＡを有するプラスミドの取得とＤＮＡの配列解析

Ｂ型肝炎ウイルス陰性でＧＰＴ値１００単位以上のヒト血清０．５ｍｌに５倍量のグアニジウムチオシアネート溶液（４Ｍグアニジウムチオシアネート、５０ｍＭ Ｔｒｉｓ－ＨＣｌ（ｐＨ７．６）、１０ｍＭ ＥＤＴＡ、０．１Ｍ ２－メルカプトメタノール、２％ザルコシル）を加え、フェノール／クロロホルム抽出し、グリコーゲンをキャリアーとしてエタノール沈澱により血清中の全ＲＮＡを精製した。

【００１９】岡本ら（Ｊａｐａｎ Ｊ．Ｅｘｐ．Ｍｅｄ．，６０巻、第３号、第１６７－１７７頁、１９９０年）の方法に従ってランダムヘキサマーをプライマーとして先に得られたＲＮＡのｃＤＮＡを、ｃＤＮＡ合成システム（ベーリンガーマンハイム社製）を用いて作製した。このｃＤＮＡをテンプレートとして、ＰＣＲ法を行い、目的とするＤＮＡ断片を増幅した。増幅されたＤＮＡ断片の５’末端をＴ４ポリヌクレオチドキナーゼによりリン酸化した後、制限酵素ＳｍａＩで消化したｐＵＣ１８と連結してクローニングした。得られた各プラスミドにクローニングされたHCVのｃＤＮＡの塩基配列は、Ｓｅｑｕｅｎａｓｅシーケンスキット（Ｕｎｉｔｅ

ｄ　Ｓｔａｔｅｓ　Ｂｉｏｃｈｅｍｉｃａｌ社製）により決定した。この結果、得られた塩基配列から目的とするＮＳ３　ＤＮＡとＮＳ４　ＤＮＡをプラスミドｐＵＣ１８のＳｍａＩサイトに、常法にしたがって挿入し、プラスミドｐＩＢ（４９８９ｂｐ）を構築した。このプラスミドに挿入されているＨＣＶの抗原タンパク質ＤＮＡの塩基配列と対応するアミノ酸配列は、配列番号１に記載されたものである。

【００２０】（参考例２）ＭＢＰ　ＤＮＡとＮＳ３およびＮＳ４タンパク質ＤＮＡとが連結した融合タンパク質の製造

上記参考例１で構築したプラスミドｐＩＢ中の抗原タンパク質ＤＮＡの５’側にＤＮＡ断片（5’ GTTGCGGAATTCG TGGACTTC）を挿入して、ＥｃｏＲＩサイトを付加した。また、同様に抗原タンパク質ＤＮＡの３’側にＤＮＡ断片（5’ ACGCGCCGAAGCTTAGTCGCTC）を挿入して、終止コドンとＨｉｎｄＩＩＩサイトを付加した。このようにして得られた４９５５ｂｐのプラスミドをｐＩＢ’と命名した。

【００２１】このｐＩＢ’を制限酵素ＥｃｏＲＩとＨｉｎｄＩＩＩとで消化して、両端が改変された抗原タンパク質ＤＮＡの断片（２２５０ｂｐ）を得た。一方、ＭＢＰを有するプラスミドｐＭＡＬ－ｃＲＩ（Ｎｅｗ　ＥｎｇｌａｎｄＢｉｏｌａｂｓ社製）を制限酵素ＥｃｏＲＩとＨｉｎｄＩＩＩとで消化して、ＭＢＰ　ＤＮＡを含む６１０１ｂｐの断片を得た。改変された抗原タンパク質ＤＮＡの断片とＭＢＰ　ＤＮＡを含むＤＮＡ断片とをＤＮＡライゲースにより連結し、得られたプラスミドをｐＭＡＬ－ＩＢと命名した。このプラスミドは、配列番号１に示されるＤＮＡの第１９番目～第２２６５番目のＤＮＡの５’側にＭＢＰ　ＤＮＡのｍａｌＥ△２－２６（大腸菌由来のマルトース結合タンパク質の第２～２６番目のアミノ酸（シグナルペプチド）が欠損したタンパク質の遺伝子）が連結したＤＮＡ領域を有するものである。

【００２２】このようにして得られたｐＭＡＬ－ＩＢで大腸菌ＪＭ１０９株を形質転換し、アンピシリン耐性の形質転換菌を得た。この形質転換菌を５０μｇ／ｍｌのアンピシリンを添加したＬＢ培地（”Ｍｏｌｅｃｕｌａｒ　Ｃｌｏｎｉｎｇ”６８頁）８ｌに６６０ｎｍでの濁度が０．１５になるように植菌した。これを３７℃で２時間振とう培養後、１ｍＭイソプロピル－β－Ｄ（－）－チオガラクトピラノシドで誘導し、さらに３７℃で４時間振とう培養した。１５，０００×ｇの遠心分離によって２０ｇの湿菌体を得た。これを６０ｍｌのトリス塩酸緩衝液（２５ｍＭ、ｐＨ８．０）に懸濁後、フレンチプレスで破砕し、１５，０００×ｇ、２０分の遠心分離で上清を集めた。これをＤＥＡＥ－トヨパール（東ソー社製）のカラム（直径２．５ｃｍ×高さ３６ｃｍ）に吸着させ、０Ｍ－０．８Ｍ　ＮａＣｌの濃度勾配（８００ｍｌ）で溶出を行い、目的とするタンパク質ＭＡＬ－ＩＢを得た。更に、アミロースレジン（Ｎｅｗ　Ｅｎｇｌａｎｄ　Ｂｉｏｌａｂｓ社製）のカラム（直径２．５ｃｍ×高さ１５ｃｍ）に吸着させ、１０ｍＭのマルトース（ＰＢＳ溶液）で溶出を行い、純度が９９％以上の目的とする融合タンパク質を約４０ｍｇ得た。この溶出されたタンパク質溶液の濃度は、ローリー法により測定したところ１．０ｍｇ／ｍｌであった。この液を抗原原液として、これ以後の操作に用いた。

【００２３】（参考例３）コアタンパク質の製造

特開平５－７８３９５号公報実施例１に記載されたＮＡＮＢＶ抗原タンパク質をコードする遺伝子を有するプラスミドｐＩＫ４ＣＥを制限酵素ＮｄｅＩとＣｌａＩとで切断後、クレノウフラグメントで平滑末端とし、０．３７ＫｂｐのＤＮＡ断片をアガロースゲルから回収した。このＤＮＡ断片は、ＨＣＶのコアタンパク質のＮ末端側１２３アミノ酸分をコードするものであり、特開平５－７８３９５号公報の配列番号１に記載された配列の第１番目から１２３番目のアミノ酸配列に相当する塩基配列である。プラスミドｐＫＫ２２３－３（ファルマシア社製）を制限酵素ＥｃｏＲＩで切断後、クレノウフラグメントで平滑末端とし、先に回収した０．３７ｋｂｐのＤＮＡ断片をＤＮＡライゲースを用いて連結し、得られたプラスミドで大腸菌ＪＭ１０９株を形質転換し、出現したアンピシリン耐性の形質転換菌から、０．３７ＫｂｐのＤＮＡ断片が正方向に挿入されているプラスミドｐＫＫＣｌａを有するコロニーを選択した。プラスミドｐＫＫＣｌａは、制限酵素ＫｐｎＩとＨｉｎｄＩＩＩとで切断したとき、約０．３ＫｂｐのＤＮＡ断片が出現するプラスミドである。

【００２４】ついで５０μｇ／ｍｌのアンピシリンを添加したＬＢ培地８ｌに、プラスミドｐＫＫＣｌａで形質転換した大腸菌ＪＭ１０９’を６６０ｎｍでの濁度が０．１５となるように植菌した。これを３７℃で２時間振とう培養した後、１ｍＭイソプロピル－β－Ｄ（－）－チオガラクトピラノシドで誘導し、さらに３７℃で２時間振とう培養した。１５，０００×ｇの遠心分離によって２０ｇの湿菌体を得た。これを６０ｍｌのＰＢＳ（１０ｍＭリン酸カリウム緩衝液、０．８５％塩化ナトリウム）に懸濁後、フレンチプレスで破砕し、１５０００×ｇ、２０分間遠心分離し、沈殿を集めた。次にこの沈殿を６０ｍｌの可溶化緩衝液（７Ｍ尿素、２０ｍＭジスレイトール、１％トリトンＸ－１００、５０ｍＭトリス塩酸（ｐＨ８．０））に懸濁し、超音波破砕機で分散後、室温で一晩振とうさせ、可溶画分として粗タンパク質液を得た。この粗タンパク質液をＣＭ－トーヨーパル（東ソー社製）のカラム（直径２ｃｍ×高さ１６ｃｍ）に吸着させ、６Ｍ尿素存在下で、０．２Ｍ－０．８Ｍ塩化ナトリウム水溶液の濃度勾配（４００ｍｌ）で溶出を行い、純度が９９％以上のＨＣＶのコアタンパク質を約

１０mg得た。これをＰＢＳで透析した後、コアタンパク質の濃度をローリー法で測定したところ、１．５mg／ｍｌであった。この液を抗原原液とした。

【００２５】（参考例４）ＭＢＰとＮＳ３タンパク質とが連結した融合タンパク質の製造

参考例１で得たプラスミドｐＩＢ（４９８９ｂｐ）をテンプレートとしてプライマー（５）5′ GTTGCGGAATTCGTGGACTTCとプライマー（６）5′ GCGAAGCTTTTAGGACTGTCTGAとを用いて常法によりＰＣＲ法を行い、８３４ｂｐのＤＮＡ断片を得た。参考例２と同様にｐＭＡＬ－ｃＲＩを制限酵素ＥｃｏＲＩとＨｉｎｄＩＩＩとで消化して、ＭＢＰ　ＤＮＡを含む６１０１ｂｐの断片を得た。先に得られた８３４ｂｐのＤＮＡの断片を制限酵素ＥｃｏＲＩとＨｉｎｄＩＩＩで消化した後、ＭＢＰ　ＤＮＡを含む６１０１ｂｐのＤＮＡ断片とＤＮＡライゲースにより連結し、得られたプラスミドをｐＩＢ３と命名し、このプラスミドｐＩＢ３で大腸菌ＪＭ１０９株を形質転換した。ついで５０μｇ／ｍｌのアンピシリンを添加したＬＢ培地８ｌに、この形質転換菌を６６０ｎｍでの濁度が０．１５となるように植菌した。これを３７℃で２時間振とう培養した後、１mMイソプロピル－β－Ｄ（－）－チオガラクトピラノシドで誘導し、さらに３７℃で２時間振とう培養した。１５，０００×ｇの遠心分離によって２５ｇの湿菌体を得た。これを７５ｍｌのＰＢＳ（１０mMリン酸カリウム緩衝液、０．８５％塩化ナトリウム）に懸濁後、フレンチプレスで破砕し、２０００×ｇ、３０分間遠心分離し、上清を得た。この上清をＤＥＡＥ－トヨパール（東ソー社製）のカラム（２．５ｃｍ×３６ｃｍ）に吸着させ、０－０．６M塩化ナトリウム水溶液の濃度勾配（６００ｍｌ）で溶出を行い、目的とするタンパク質ＭＡＬ－ＩＢ３（ＭＢＰのＣ末端側にＮＳ３タンパク質が連結している融合タンパク質）を得た。更に、アミロースレジン（New England Biolabs社製）のカラム（直径２．５ｃｍ×高さ１５ｃｍ）に吸着させ、１０mMのマルトース（ＰＢＳ溶液）で溶出を行い、純度が９９％以上のタンパク質ＭＡＬ－ＩＢ３（７５mg）を得た。タンパク質溶液の濃度をローリー法で測定したところ、２．５mg／ｍｌであった。この液を抗原原液とした。

【００２６】（参考例５）ＮＳ３タンパク質の製造

参考例４で得られた抗原原液３０ｍｌ（ＭＡＬ－ＩＢ３融合タンパク質量は７５mg）をファクターＸａ溶液（New England Biolabs社製）を２５０μｌと1Mの塩化カルシウム水溶液１２０μｌを加え、室温で一晩静置した。その後、これに塩化ナトリウムを溶解させて塩化ナトリウムの濃度が２Mとなるように調製した。これをフェニル－トヨパール（東ソー社製）のカラム（直径１．５ｃｍ×高さ１０ｃｍ）に吸着させた。２M－０M塩化ナトリウムの濃度勾配（１００ｍｌ）で溶出を行い、純度９９％以上のタンパク質ＩＢ３を得た。このタンパク質溶液をローリー法で測定したところ、１．８ｍｌ／ｍｌであった。この液を抗原原液とした。

【００２７】（参考例６）ＭＢＰとＮＳ４タンパク質とが連結した融合タンパク質の製造

参考例１で得たプラスミドｐＩＢ（４９８９ｂｐ）をテンプレートとしてプライマー（７）5′ CTCACTGAATTCGATGCCCACとプライマー（８）5′ ACGCGCCGAAGCTTAGTCGCTとを用いて常法によりＰＣＲ法を行い、１１３７ｂｐのＤＮＡ断片を得た。参考例２と同様にｐＭＡＬ－ｃＲＩを制限酵素ＥｃｏＲＩとＨｉｎｄＩＩＩとで消化して、ＭＢＰ　ＤＮＡを含む６１０１ｂｐの断片を得た。先に得られた１１３７ｂｐのＤＮＡの断片を制限酵素ＥｃｏＲＩとＨｉｎｄＩＩＩで消化した後、ＭＢＰ　ＤＮＡを含む６１０１ｂｐのＤＮＡ断片とＤＮＡライゲースにより連結し、得られたプラスミドをｐＩＢ４と命名し、このプラスミドｐＩＢ４で大腸菌ＪＭ１０９株を形質転換した。ついで５０μｇ／ｍｌのアンピシリンを添加したＬＢ培地７．５ｌに、この形質転換菌を６６０ｎｍでの濁度が０．１５となるように植菌した。これを３７℃で２時間振とう培養した後、１mMイソプロピル－β－Ｄ（－）－チオガラクトピラノシドで誘導し、さらに３７℃で２時間振とう培養した。１５，０００×ｇの遠心分離によって２０ｇの湿菌体を得た。これを６０ｍｌのＰＢＳ（１０mMリン酸カリウム緩衝液、０．８５％塩化ナトリウム）に懸濁後、フレンチプレスで破砕し、２５０００×ｇ、６０分間遠心分離し、沈殿を集めた。この沈殿を４０ｍｌの可溶化緩衝液（７M尿素、２０mMジスレイトール、１％トリトンＸ－１００、５０mMトリス塩酸（ｐＨ８．０））に懸濁し、超音波破砕機で分散後、室温で一晩振とうさせ、可溶画分として粗タンパク質液を得た。この粗タンパク質液から透析で尿素を除去した後、アミロースレジン（New England Biolabs社製）のカラム（直径２．５ｃｍ×高さ１５ｃｍ）に吸着させ、１０mMのマルトース（ＰＢＳ溶液）で溶出を行い、純度が９９％以上のタンパク質ＭＡＬ－ＩＢ４（７０mg）を得た。タンパク質溶液の濃度をローリー法で測定したところ、１．５mg／ｍｌであった。この液を抗原原液とした。

【００２８】（参考例７）アミノ酸ポリマー粒子の製造

等電点（ｐＩ）が８．８のゼラチンを水に懸濁させ、加温して溶解させた。この液を水酸化ナトリウム水溶液を用いてｐＨ８．５に調整し、水を添加して５％のゼラチン溶液を得た。また、アラビアゴムを水に溶解させ、濾過することにより４％のアラビアゴム水溶液を得た。得られた２つの水溶液を４０℃に加温して、それぞれ１０ｍｌづつ混合し、４０℃に加温した４０％メタノール水溶液６０ｍｌに注ぎ入れてよく撹拌した。続いて酢酸を滴下して、ｐＨを約４にまで下げて粒子を形成させた。得られた粒子分散液を氷冷し、０．５ｇのグルタルアル

デヒドを加えて、よく攪拌した。１昼夜、４℃で静置した後、９００×ｇで１０分間遠心して、粒子を回収した。回収した粒子は、遠心操作で充分水で洗浄した後、４％ホルムアルデヒド水溶液に分散し、４℃で５日間静置した。これを９００×ｇで遠心して、粒子を回収し、遠心操作で水で洗浄した。このようにして得られた粒子の数平均粒子径は約４μｍであった。

【００２９】（実施例１）抗原感作粒子Ａの調製
参考例７で得た粒子（以下、担体粒子という）に、水を加えて１０％分散液を調整し、参考例２で得た抗原原液に、リン酸緩衝液を加えて５０μｇ／ｍｌに希釈した希釈液を調整した。１０％担体粒子分散液０．５ｍｌに抗原の５０μｇ／ｍｌ希釈液１ｍｌと５０ｍｇ／ｍｌに調整した１－エチル－３－（３－ジメチルアミノプロリル）カルボジイミド水溶液０．０５ｍｌを加えた。これを３７℃で１．５時間振盪した後、先に調製した粒子懸濁用液（０．１Ｍリン酸カリウム緩衝液（ｐＨ６．５）・１％正常ウサギ血清・０．０５％NaN_3）２ｍｌで３回洗浄し、最後に４ｍｌの粒子懸濁用液に懸濁して１．２５％の抗原感作粒子Ａを得た。

【００３０】（実施例２）抗原感作粒子Ｂの調製
参考例２および３で得た抗原原液をそれぞれ２０、１０μｇ／ｍｌつづ用いる以外は実施例１と同様の方法により１．２５％の抗原感作粒子Ｂを得た。

【００３１】（実施例３）抗原感作粒子Ｃの調製
参考例３、４および６で得た抗原原液をそれぞれ２０、１０、１０μｇ／ｍｌつづ用いる以外は実施例１と同様の方法により１．２５％の抗原感作粒子Ｃを得た。

【００３２】（実施例４）抗原感作粒子Ｄの調製
参考例３、５および６で得た抗原原液をそれぞれ２０、５、１０μｇ／ｍｌつづ用いる以外は実施例１と同様の方法により１．２５％の抗原感作粒子Ｄを得た。

【００３３】（試験例）マイクロタイター法によるＨＣＶ感染患者血清との反応性の検討
診断薬粒子として実施例１で得た感作粒子Ａ、Ｂ、Ｃ、およびＤとオーソ・ダイアグノスティック・システムズ社製の商品名「オーソ　ＨＣＶ　Ａｂ　ＰＡテストＩＩ」の粒子（コアタンパク質、ＮＳ３タンパク質、ＮＳ４タンパク質の一部から成るＨＣＶ抗原タンパク質をゼラチン粒子に結合させているもの；以下、表を含めて「市販品」という）を用い、オーソ・ダイアグノスティック・システムズ社製のオーソ　ＨＣＶ　Ａｂ　ＰＡテストＩＩの定量法マニュアルに従って、それぞれの粒子を用いた場合のマイクロタイター法による試験を行った。表中＋は凝集が観察されたもの、－は凝集が観察されなかったものである。　結果は表１に示す。

【００３４】
【表１】

Ｃ型肝炎患者血清の希釈度	抗原感作粒子Ａ	抗原感作粒子Ｂ	抗原感作粒子Ｃ	抗原感作粒子Ｄ	比較例 市販品
1／8192	＋	＋	＋	＋	＋
1／16384	＋	＋	＋	＋	＋
1／32768	＋	＋	＋	＋	－
1／65536	＋	＋	＋	＋	－
1／131072	＋	＋	＋	＋	－
1／262144	－	＋	＋	＋	－
1／524288	－	－	－	－	－

【００３５】表１の結果から、本発明の抗原感作粒子は、市販品と比べて診断薬としての鋭敏性が優れていることが分かった。なお、各抗原感作粒子・比較例ともに対照実験として検体希釈液の代わりに正常人血清を用いたところ、凝集像はいづれの血清希釈倍率においても全く観察されなかった。

【００３６】配列番号:1
配列の長さ:2303
配列の型:核酸
鎖の数:二本鎖
トポロジー:直鎖状
配列の種類:Genomic DNA
起源
生物名:血液伝播型非Ａ非Ｂ型肝炎ウイルス

配列

```
TGC ACC CGG GGG GTT GCG AAG GCG GTG GAC TTC ATA CCC GTT GAG CCT      48
Cys Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Pro
 1               5                  10                  15
ATG GAA ACT ACT ATG CGG TCT CCG GTC TTC ACA GAC AAC TCT TCC CCC      96
```

```
      13                                                          14
Met Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro
                20                  25                  30
CCG GCT GTA CCG CAG ACA TTC CAA GTG GCC CAT CTA CAC GCT CCC ACT     144
Pro Ala Val Pro Gln Thr Phe Gln Val Ala His Leu His Ala Pro Thr
        35                  40                  45
GGC AGC GGT AAG AGC ACC AGA GTG CCA GCT GCA TAT GCC AGC CAA GGG     192
Gly Ser Gly Lys Ser Thr Arg Val Pro Ala Ala Tyr Ala Ser Gln Gly
    50                  55                  60
TAC AAG GTG CTC GTC TTG AAC CCG TCC GTT GCC GCC ACA TTG GGC TTT     240
Tyr Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe
65                  70                  75                  80
CGG GCG TAT ATG TCT AAA GCA CAT GGT ATC GAC CCC AAC ATC AGA ACT     288
Arg Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr
                85                  90                  95
GGG GTG AGG ACT ATC ACC ACG GGT GCC CCT ATC ACA TAC TCC ACC TAC     336
Gly Val Arg Thr Ile Thr Thr Gly Ala Pro Ile Thr Tyr Ser Thr Tyr
            100                 105                 110
GGC AAG TTC CTT GCC GAC GGT GGA TGC TCC GGG GGC GCC TAT GAC ATC     384
Gly Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile
        115                 120                 125
ATC ATA TGT GAT GAG TGC CAC TCA ACT GAC TCA ACT ACC ATC TTG GGC     432
Ile Ile Cys Asp Glu Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly
    130                 135                 140
ATT GGC ACA GTC CTG GAC CAA GCG GAG ACG GCT GGA GCT CGG CTC GTC     480
Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val
145                 150                 155                 160
GTG CTC GCC ACC GCT ACG CCT CCG GGA TCG GTC ACC GTA CCA CAC CCC     528
Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro
                165                 170                 175
AAT ATC GAG GAG GTG GCC CTG TCC AAC ACA GGA GAG ATT CCC TTC TAC     576
Asn Ile Glu Glu Val Ala Leu Ser Asn Thr Gly Glu Ile Pro Phe Tyr
            180                 185                 190
GGC AAA GCC ATC CCC ATC GAG GTC ATC AAG GGG GGA AGT CAT CTC ATT     624
Gly Lys Ala Ile Pro Ile Glu Val Ile Lys Gly Gly Ser His Leu Ile
        195                 200                 205
TTC TGC CAT TCC AAG AAG AAG TGT GAC GAG CTC GCC GCA AAG CTG TCA     672
Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Ser
    210                 215                 220
GCC CTC GGA CTC AAT GCT GTA GCG TAT TAT CGG GGT CTT GAT GTG TCC     720
Ala Leu Gly Leu Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser
225                 230                 235                 240
GTC ATA CCG ACC AGC GGA GAC GTC GTC GTC GTG GCG ACA GAC GCT CTA     768
Val Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu
                245                 250                 255
ATG ACG GGC TAC ACC GGC GAC TTT GAC TCA GTG ATC GAC TGT AAC ACA     816
Met Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr
            260                 265                 270
TGT GTC ATC CAG ACA GTC GAT TTT AGT TTG GAT CCC ACT TTC ACC ATC     864
Cys Val Ile Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile
        275                 280                 285
```

15　　16

```
GAG ACA ACG ACC GTG CCC CAA GAC GCG GTG TCG CAC CCG CAA CGG CGA     912
Glu Thr Thr Thr Val Pro Gln Asp Ala Val Ser His Pro Gln Arg Arg
    290                 295                 300
GGT AGG ACT GGC AGA GGT AGG AGA GGC ATC TAC AGG TTT GTG ACT CCA     960
Gly Arg Thr Gly Arg Gly Arg Arg Gly Ile Tyr Arg Phe Val Thr Pro
305                 310                 315                 320
GGA GAA CGG CCC TCG GGC ATG TTC GAT TCT TCG GTC CTG TGT GAG TGC    1008
Gly Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys
                325                 330                 335
TAT GAC GCA GGC TGT GCT TGG TAC GAG CTC ACG CCC GCT GAG ACT TCA    1056
Tyr Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Ser
            340                 345                 350
GTT AGG TTA CGG GCT TAC CTG AAT ACA CCA GGT TTA CTC GTC TGT CAG    1104
Val Arg Leu Arg Ala Tyr Leu Asn Thr Pro Gly Leu Leu Val Cys Gln
        355                 360                 365
GAC CAT CTG GAG TTC TGG GAG GGT GTC TTC ACA GGC CTC ACT CAT ATA    1152
Asp His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile
    370                 375                 380
GAT GCC CAC TTC TTG TCT CAG ACT AAG CAA GCA GGA GAC AGC TTC CCC    1200
Asp Ala His Phe Leu Ser Gln Thr Lys Gln Ala Gly Asp Ser Phe Pro
385                 390                 395                 400
TAC CTG GTA GCA TAC CAG GCT ACA GTG TGC GCC AGG GCC CAA GCT CTA    1248
Tyr Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Leu
                405                 410                 415
CCT CCA TCG TGG GAT CAA ATG TGG AAG TGT CTC ACA CGG CTA AAG CCT    1296
Pro Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Thr Arg Leu Lys Pro
            420                 425                 430
ACG CTA ACG CGG CCA ACA CCC CTG TTG TAT AGG CTA GGA GCT GTG CAA    1344
Thr Leu Thr Arg Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln
        435                 440                 445
AAC GAG GTC ACC CTC ACA CAC CCC GTA ACC AAA TAC ATC ATG GCA TGC    1392
Asn Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Ala Cys
    450                 455                 460
ATG TCA GCT GAC CTA GAG ATC GTC ACT AGC ACC TGG GTG CTG GTA GGC    1440
Met Ser Ala Asp Leu Glu Ile Val Thr Ser Thr Trp Val Leu Val Gly
465                 470                 475                 480
GGG GTC CTT GCC GCT CTG GCC GCG TAC TGC CTG ACA ACG GGC AGC GTG    1488
Gly Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Thr Thr Gly Ser Val
                485                 490                 495
GTC ATT GTG GGC AGG GTC GTC TTG TCA GGG AGG CCG GCT ATC ATT CCC    1536
Val Ile Val Gly Arg Val Val Leu Ser Gly Arg Pro Ala Ile Ile Pro
            500                 505                 510
GAC AGG GAA GTT CTC TAC CGG GAG TTC GAC GAG ATG GAG GAG TGC GCC    1584
Asp Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys Ala
        515                 520                 525
ACA CAC CTC CCT TAC ATC GAA CAG GGA ATG CAG CTC GCC GAG CAA TTC    1632
Thr His Leu Pro Tyr Ile Glu Gln Gly Met Gln Leu Ala Glu Gln Phe
    530                 535                 540
AAG CAG AAG GCG TTC GGG TTG CTG CAA ACA GCC ACC AAA CAA GCG GAG    1680
Lys Gln Lys Ala Phe Gly Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu
```

17　　18

```
545                 550                 555                 560
GCT GCT GCT CCC GTG GTG GAG TCC AAG TGG CGG ACC CTT GAG GCT TTC    1728
Ala Ala Ala Pro Val Val Glu Ser Lys Trp Arg Thr Leu Glu Ala Phe
                565                 570                 575
TGG GCG AAG CAC ATG TGG AAT TTC ATC AGC GGG ATA CAA TAC TTG GCG    1776
Trp Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala
            580                 585                 590
GGC CTG TCG ACT CTG CCT GGG AAC CCC GCG ATA GCA TCG CTC ATG GCA    1824
Gly Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala
        595                 600                 605
TTC ACA GCC TCT ATC ACC AGC CCG CTC ACC ACC CAA CAC ACC CTC TTG    1872
Phe Thr Ala Ser Ile Thr Ser Pro Leu Thr Thr Gln His Thr Leu Leu
    610                 615                 620
TTT AAC ATC TTG GGG GGA TGG GTG GCT GCC CAA CTC GCC CCC ACC AGC    1920
Phe Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Pro Thr Ser
625                 630                 635                 640
GCT GCT TCA GCT TTC GTG GGC GCC GGC ATT GCC GGT GCG GCT GTT GGC    1968
Ala Ala Ser Ala Phe Val Gly Ala Gly Ile Ala Gly Ala Ala Val Gly
                645                 650                 655
AGC ATA GGC CTT GGG AAG GTG CTT GTG GAC ATT CTA GCG GGT TAT GGA    2016
Ser Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly
            660                 665                 670
GCG GGG GTG GCA GGC GCA CTC GTG GCC TTT AAG GTC ATG AGT GGT GAG    2064
Ala Gly Val Ala Gly Ala Leu Val Ala Phe Lys Val Met Ser Gly Glu
        675                 680                 685
ATG CCC TCC ACT GAG GAC CTG GTC AAC TTG CTC CCT GCT ATC CTC TCT    2112
Met Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Ser
    690                 695                 700
CCT GGT GCC CTG GTC GTC GGG GTC GTG TGC GCA GCA ATA CTG CGT CGG    2160
Pro Gly Ala Leu Val Val Gly Val Val Cys Ala Ala Ile Leu Arg Arg
705                 710                 715                 720
CAT GTG GGC CCA GGG GAG GGG GCT GTG CAG TGG ATG AAC CGG CTG ATA    2208
His Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile
                725                 730                 735
GCG TTC GCT TCG CGG GGC AAC CAC GTC TCC CCC ACG CAC TAT GTG CCT    2256
Ala Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val Pro
            740                 745                 750
GAG AGC GAC GCC GCA GCG CGC GTC ACC CAG ATC CTC TCC AGC CTT AC     2303
Glu Ser Asp Ala Ala Ala Arg Val Thr Gln Ile Leu Ser Ser Leu
        755                 760                 765     767
```

フロントページの続き

(51) Int. Cl.[6]	識別記号	庁内整理番号	F I	技術表示箇所
//(C12N 15/09	ZNA			
C12R 1:92)				
(C12P 21/02				
C12R 1:19)				

C12R 1:92)